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(54) NF-kB activity inhibitor

(57) This invention relates to an NF-κB activity inhibitor which contains alkaloids originated from a plant belonging to the genus *Stephania* of the family *Menspermaceae*, derivatives thereof and salts thereof, as the active components, to an agent for use in the treatment and prevention of diseases upon which the NF-κB activity inhibiting action is effective and to an inhibitor of the expression of related genes. Since said active components exert an action to inhibit transcription of DNA having an NF-κB recognition sequence by inhibiting the activity of NF-κB, the drug of the present invention can inhibit expression of genes of certain substances such as cytokines, inflammatory cytokine receptor antagonists, MHC class I, MHC class II, β2 microglobulin, immunoglobulin light chain, serum amyloid A, angiotensinogen, complement B, complement C4, C-myc gene, HIV, SV40, CMV, adenovirus and the like, so that the inventive drug is useful in treating and/or preventing various diseases in which these substances are taking roles.

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Description

FIELD OF THE INVENTION

[0001] This invention relates to an NF-κB activity inhibitor, to agents for use in the treatment and prevention of diseases upon which NF-κB activity inhibiting action is effective, particularly an agent for the treatment and prevention of inflammatory diseases, an agent for the treatment and prevention of autoimmune diseases and an agent for the treatment and prevention of viral diseases, and to a gene expression inhibitor.

BACKGROUND OF THE INVENTION

[0002] DNA as the substance of genes is regulated by various factors, and expression of its genetic information is controlled thereby. For example, transcription of genetic information from DNA to RNA is controlled by a plurality of DNA binding proteins which recognizes several to scores of nucleotide sequences on the gene and bind thereto. NF-κB (nuclear factor-κB) known as one of such DNA binding proteins is present in the nuclear extract of B cells which are antibody-producing cells and has been identified as a factor that binds to the enhancer of immunoglobulin κ chain (Igκ) gene. With the progress of studies on this factor, it has been revealed that this is a transcription factor which takes part in the expression induction of a large number of genes that are induced by stimulation and is broadly concerned in the regulation of vital phenomenon.

[0003] This NF-κB is generally present in the cytoplasm in the form of a complex in which its homodimer of proteins having a molecular weight of 50 kD or its heterodimer of a protein of 50 kD in molecular weight and a protein of 65 kD in molecular weight is bonded to a protein called I-κB which inhibits activity of the dimer. When a certain stimulation is given to the cells, I-κB is modified and released from the complex to cause activation of NF-κB, so that the dimer is transferred into the nucleus and its DNA binding activity becomes detectable. It is known that this activity is generated as a result of direct activation, not mediated by the expression of other genes such as second messengers and the like.

[0004] In addition, the NF-κB binding sequence on DNA has been found in various genes and it has been shown that it is actually important for the expression of the function of genes. The binding sequence of NF-κB (κB motif) is composed of about 10 bases having a common sequence which starts with a cluster of G (guanine) and ends with a cluster of C (cytosine) (consensus sequence 5'-GGGRNNYCCC-3'). However, a number of sequences to which DNA binding proteins can be bonded are present on the genes of interleukin-1 (to be referred to as IL-1 hereinafter in some cases) and tumor necrosis factor (to be referred to as TNF hereinafter in some cases) which are known as inflammatory proteins, and it is known that the NF-κB binding sequence is also present therein (Clark, B.D. *et al.*, *Nucl. Acids Res.*, 14, 7898, 1984; Nedospasov, S.A. *et al.*, *Cold Spring Harb. Symp. Quant. Biol.*, 51, 611, 1986). Actually, it has been reported that the binding of NF-κB inhibits transcription to mRNA (Hiscott, J. *et al.*, *Mol. Cell. Biol.*, 13, 6231, 1993; Collart, M.A. *et al.*, *Mol. Cell. Biol.*, 10, 1498, 1990).

[0005] As a substance which inhibits the transcription factor of NF-κB, an NF-κB binding protein has been disclosed in European Patent 584238.

[0006] In addition, it has been reported that a composition which contains an alkaloid originated from a plant belonging to the genus *Stephania* of the family *Menspermaceae*, as its active ingredient, inhibits production of TNFα, interleukin-6 (to be referred to as IL-6 hereinafter in some cases) and interleukin-8 (to be referred to as IL-8 hereinafter in some cases) (JP-A-8-301761, the term "JP-A" as used herein means an "unexamined published Japanese patent application").

[0007] Phospholipid which constitutes the biological membrane releases arachidonic acid by the action of phospholipase A_2. Leukotriene, thromboxane, prostaglandine and the like are produced from the arachidonic acid by the action of 5-lipoxygenase or cyclooxygenase. These substances exert complex physiological activities and take important roles in the maintenance and regulation of the living body. In the living body, various cytokines are released by receiving various types of stimulation and cause inflammatory reactions. The prior art drugs inhibit expression of histamine and leukotriene B4 or prostaglandine E2 or the like inflammatory protein by the antagonism on mediator receptors of histamine and the like or by the inhibition of lipoxygenase, cyclooxygenase and the like metabolic enzymes in the arachidonic acid cascade. However, effects of non-steroidal drugs are expected for only symptomatic therapy and not sufficient as radical therapy, while steroid drugs are effective but have a problem in that they cannot be administered for a prolonged period of time due to their strong side effects. Particularly, autoimmune disease and the like inflammatory diseases become chronic in many cases and therefore require prolonged medical treatments, so that drugs having side effects are not applicable to such diseases. In addition, NF-κB takes an important role in the replication of HIV-1, so that search for a substance capable of inhibiting NF-κB activity is expected for not only its anti-inflammatory effects but also inhibition of acquired immunodeficiency syndrome (AIDS and the like) by its effect to inhibit transcription of long terminal repeat (LTR) of HIV-1, namely replication of the virus.

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SUMMARY OF THE INVENTION

[0008] In view of the above, it therefore becomes an object of the present invention to provide an NF-κB activity inhibitor. Another object of the present invention is to provide an agent for the treatment and prevention of diseases upon which NF-κB activity inhibiting action is effective. Sill another object of the present invention is to provide an inhibitor of the expression of genes based on the NF-κB activity inhibiting action.

[0009] The inventors of the present invention have conducted intensive studies on the methods and substances which can radically inhibit various inflammatory cytokines and found as the results that alkaloids originated from a plant belonging to the genus *Stephania* of the family *Menspermaceae*, derivatives thereof and salts thereof can inhibit various cytokines and the like at the gene level based on their transcription factor NF-κB activity inhibiting action and have an activity to inhibit transcription of HIV-1 LTR. The present invention has been accomplished on the basis of these findings.

[0010] Accordingly, the present invention relates to an NF-κB activity inhibitor which comprises, as its active ingredient, at least one compound selected from the group consisting of alkaloids originated from a plant belonging to the genus *Stephania* of the family *Menspermaceae*, derivatives thereof and salts thereof; to an agent for the treatment and prevention of diseases upon which NF-κB activity inhibiting action is effective (particularly an agent for the treatment and prevention of inflammatory diseases, an agent for the treatment and prevention of autoimmune diseases and an agent for the treatment and prevention of viral diseases); and to an gene expression inhibitor.

[0011] The present invention also relates to a method for inhibiting NF-κB activity which comprises using at least one compound selected from the group consisting of alkaloids originated from a plant belonging to the genus *Stephania* of the family *Menspermaceae*, derivatives thereof and salts thereof; to a method for the treatment and prevention of diseases upon which NF-κB activity inhibiting action is effective (particularly for the treatment and prevention of inflammatory diseases, for the treatment and prevention of autoimmune diseases and or the treatment and prevention of viral diseases); and to a method for inhibiting expression of genes.

[0012] Moreover, the present invention relates to use of at least one compound selected from the group consisting of alkaloids originated from a plant belonging to the genus *Stephania* of the family *Menspermaceae*, derivatives thereof and salts thereof, for the manufacture of an NF-κB activity inhibitor; use thereof for the manufacture of an agent for the treatment and prevention of diseases upon which NF-κB activity inhibiting action is effective (particularly an agent for the treatment and prevention of inflammatory diseases, an agent for the treatment and prevention of autoimmune diseases and an agent for the treatment and prevention of viral diseases); and to use thereof for the manufacture of an gene expression inhibitor.

DETAILED DESCRIPTION OF THE INVENTION

[0013] The alkaloid as the active ingredient of the present invention can be extracted in the usual way from a plant belonging to the genus *Stephania* of the family *Menspermaceae* (for example, *Stephania cepharantha Hayata, Stephania sasaki* Hayata or the like). Preferably, the alkaloid originated from *Stephania cepharantha* Hayata is used.

[0014] An extract obtained by concentrating the extract from a plant belonging to the genus *Stephania*, a precipitate which is formed when an acidic solution of the extract is alkalified and an alkaloid-containing fraction separated by this treatment, as well as crystals obtained by separating and purifying the alkaloid in the usual way and derivatives of said alkaloid produced known methods, can be used as the active ingredient of the present invention. For example, an alkaloid fraction can be separated by extracting a plant of the genus *Stephania* (its roots, stems, seeds, leaves and the like can be used, though not particularly limited to these parts) with methanol, ethanol, acetone, ethyl acetate, benzene or the like solvent, concentrating the extract, dissolving the concentrate in dilute hydrochloric acid, dilute sulfuric acid, citric acid aqueous solution, oxalic acid aqueous solution or the like acidic solution, alkalifying the solution and then collecting the thus formed precipitate. The thus obtained fraction may be further purified by various chromatography techniques, recrystallization and the like known means.

[0015] Examples of the alkaloid originated from a plant of the genus *Stephania* include cepharanthine, isotetrandrine, berbamine, cycleanine, homoaromoline, cepharanoline, aromoline, obamegine, norcycleanine, 2-norcepharanthine, 2-norcepharanoline, 2-norberbamine, secocepharanthine, obaberine, 2-norisotetrandrine, oxyacanthine, stephibaberine, thalrugosine and the like bisbenzylisoquinoline alkaloids; coclaurine, reticuline, laudanidine, protosinomenine, N-methylcoclaurine and the like benzylisoquinoline alkaloids; FK-3000, sinomenine, cephamonine, tannagine, cephamuline and the like morphinan alkaloids; lastourvilline, isocorydine, corydine and the like aporphine alkaloids; stepharine and the like proaporphine alkaloids; and cepharamine, aknadinine, aknadilactam and the like hasubanane alkaloids.

[0016] Examples of the aforementioned derivatives of said alkaloid include acyl derivatives, alkyl derivatives, carbamoyl derivatives and the like.

[0017] Examples of the acyl group in the acyl derivatives include saturated straight chain aliphatic acyl groups having 2 to 18 carbon atoms (for example, acetyl, propionyl, butyryl, valeryl, caproyl, capryloyl, lauroyl, palmitoyl, stearoyl and

the like groups), aromatic acyl groups (for example, benzoyl, 4-methoxybenzoyl, 4-chlorobenzoyl, 4-nitrobenzoyl, 3,4-dimethoxybenzoyl, 1-naphthalenecarboxy, 3-indolecarboxy and the like groups) and aryl acetate groups (for example, phenylacetyl, 4-methoxyphenylacetyl, 4-chlorophenylacetyl, 4-nitrophenylacetyl, 3,4-dimethoxyphenylacetyl, 1-naphthaleneacetyl, 3-indoleacetyl and the like groups).

[0018] Illustrative examples of the acyl derivatives include 12-O-acetylcepharanoline, 12-O-propionylcepharanoline, 12-O-butyrylcepharanoline, 12-O-valerylcepharanoline, 12-O-caproylcepharanoline, 12-O-capryloylcepharanoline, 12-O-lauroylcepharanoline, 12-O-palmitoylcepharanoline, 12-O-stearoylcepharanoline, 12-O-benzoylcepharanoline, 12-O-(4-methoxybenzoyl)cepharanoline, 12-O-(4-chlorobenzoyl)cepharanoline, 12-O-(4-nitrobenzoyl)cepharanoline, 12-O-(3,4-dimethoxybenzoyl)cepharanoline, 12-O-(1-naphthalenecarboxy)cepharanoline, 12-O-(3-indolecarboxy)cepharanoline, 12-O-phenylacetylcepharanoline, 12-O-(4-methoxyphenyl)acetylcepharanoline, 12-O-(4-chlorophenyl)acetylcepharanoline, 12-O-(4-nitrophenyl)acetylcepharanoline, 12-O-(3,4-dimethoxyphenyl)acetylcepharanoline, 12-O-(1-naphthalene)acetylcepharanoline, 12-O-(3-indole)acetylcepharanoline and the like.

[0019] Examples of the alkyl group in the alkyl derivatives include methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl and the like saturated straight chain alkyl groups having 1 to 11 carbon atoms, as well as benzyl, 3-methoxybenzyl, 3-chlorobenzyl, 1-naphthalenemethyl and the like groups.

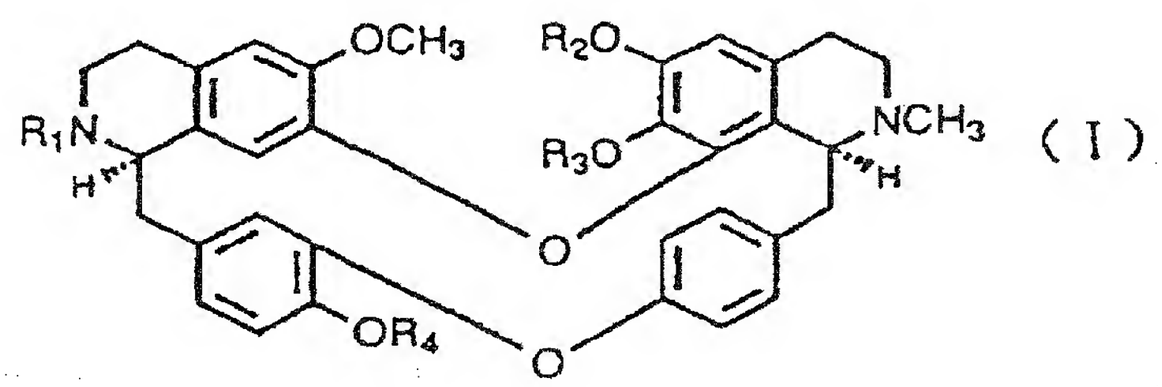
[0020] Illustrative examples of the alkyl derivatives include 12-O-methylcepharanoline, 12-O-ethylcepharanoline, 12-O-propylcepharanoline, 12-O-butylcepharanoline, 12-O-pentylcepharanoline, 12-O-hexylcepharanoline, 12-O-heptylcepharanoline, 12-O-octylcepharanoline, 12-O-nonylcepharanoline, 12-O-decylcepharanoline, 12-O-undecylcepharanoline, 12-O-benzylcepharanoline, 12-O-(3-methoxybenzyl)cepharanoline, 12-O-(3-chlorobenzyl)cepharanoline, 12-O-(1-naphthalenemethyl)cepharanoline and the like.

[0021] The carbamoyl derivatives may have a (mono or di)alkyl substituted carbamoyl group, and examples of the alkyl group as a substituent group include those which are described above, as well as cyclohexyl, benzyl, 4-methoxybenzyl, 4-chlorobenzyl, furfuryl and the like groups.

[0022] Illustrative examples of the carbamoyl derivatives include 12-O-ethylcarbamoylcepharanoline, 12-O-propylcarbamoylcepharanoline, 12-O-butylcarbamoylcepharanoline, 12-O-pentylcarbamoylcepharanoline, 12-O-hexylcarbamoylcepharanoline, 12-O-heptylcarbamoylcepharanoline, 12-O-octylcarbamoylcepharanoline, 12-O-nonylcarbamoylcepharanoline, 12-O-decylcarbamoylcepharanoline, 12-O-cyclohexylcarbamoylcepharanoline, 12-O-benzylcarbamoylcepharanoline, 12-O-(4-methoxybenzyl)carbamoylcepharanoline, 12-O-(4-chlorobenzyl)carbamoylcepharanoline, 12-O-furfurylcarbamoylcepharanoline, 12-O-diethylcarbamoylcepharanoline, 12-O-dipropylcarbamoylcepharanoline, 12-O-dibutylcarbamoylcepharanoline, 12-O-dihexylcarbamoylcepharanoline, 12-O-dioctylcarbamoylcepharanoline, 12-O-didecylcarbamoylcepharanoline and the like.

[0023] The NF-κB activity inhibitor, agent for the treatment and prevention of diseases upon which the NF-κB activity inhibiting action is effective and inhibitor of the expression of genes of the present invention may contain at least one alkaloid, a derivative thereof or a salt thereof or may contain a mixture of two or more alkaloids, derivatives thereof or salts thereof.

[0024] The aforementioned bisbenzylisoquinoline alkaloids are compounds having the following structures.



(I)

	R_1	R_2	R_3	R_4
2-Norcepharanoline	H	$-CH_2-$		H
Oxyacanthine	CH_3	$-CH_3$	CH_3	H
Stephibaberine	CH_3	H	CH_3	CH_3

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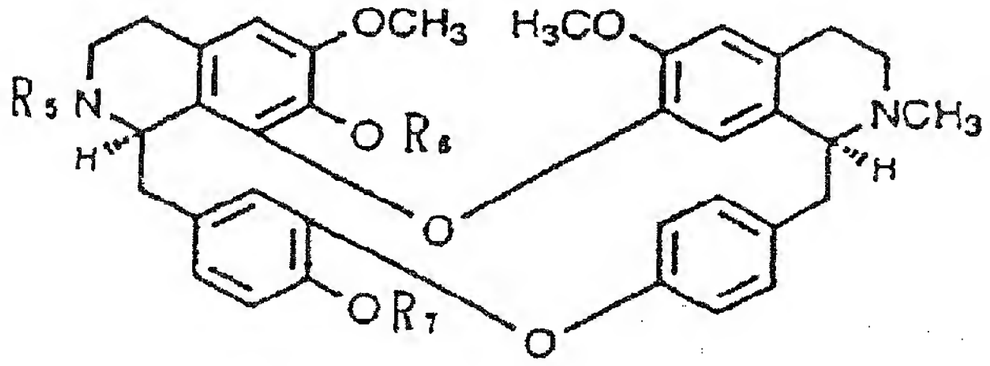
(continued)

	R_1	R_2	R_3	R_4
2-Norcepharanthine	H	-CH_2-		CH_3
Cepharanthine	CH_3	-CH_2-		CH_3
Cepharanoline	CH_3	-CH_2-		H
Obaberine	CH_3	CH_3	CH_3	CH_3
Homoaromoline	CH_3	CH_3	H	CH_3
Aromoline	CH_3	CH_3	H	H



(II)

	R_5	R_6	R_7
2-Norberbamine	H	CH_3	H
2-Norisotetrandrine	H	CH_3	CH_3
Thalrugosine	CH_3	H	CH_3
Berbamine	CH_3	CH_3	H
Isotetrandrine	CH_3	CH_3	CH_3
Obamegine	CH_3	H	H

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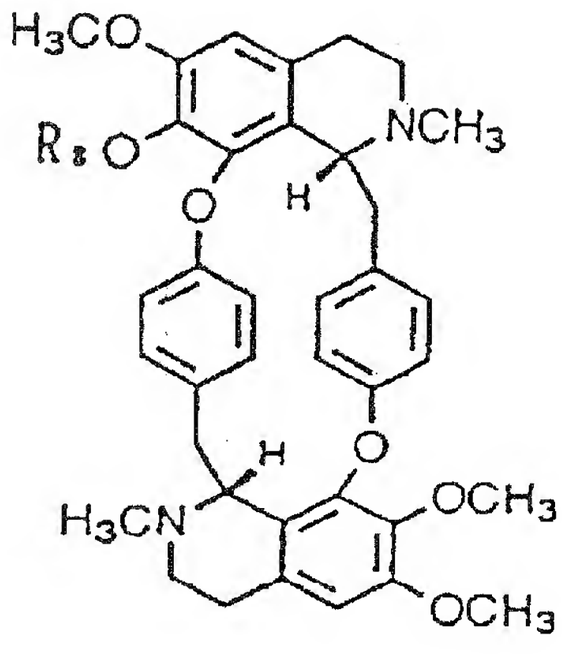
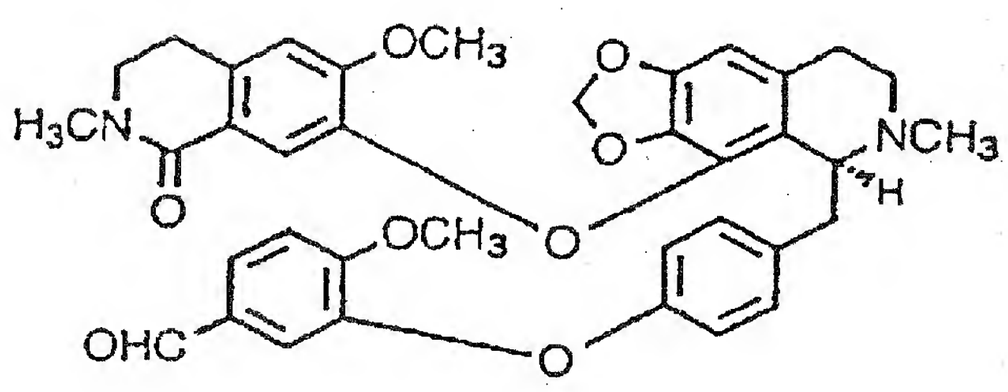


(III)

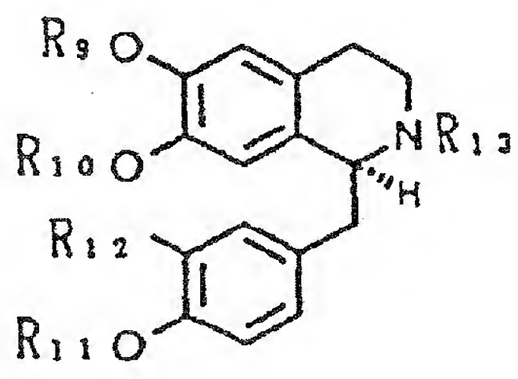
	R_8
Norcycleanine	H
Cycleanine	CH_3



(IV)

Secocepharanthine

[0025] Also, the aforementioned benzylisoquinoline alkaloids are compounds having the following structures.



(V)

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	R_9	R_{10}	R_{11}	R_{12}	R_{13}
Protosinomenine	H	CH_3	CH_3	OH	CH_3
N-Methylcoclaurine	CH_3	H	H	H	CH_3
Reticuline	CH_3	H	CH_3	OH	CH_3
Coclaurine	CH_3	H	H	H	H
Laudanidine	CH_3	CH_3	CH_3	OH	CH_3

[0026] The NF-κB activity inhibitor, agent for the treatment and prevention of diseases upon which the NF-κB activity inhibiting action is effective and inhibitor of the expression of genes of the present invention may preferably contain at least one alkaloid selected from cepharanthine, isotetrandrine, berbamine, cycleanine, homoaromoline and cepharanoline, of which cepharanthine is more preferred.

[0027] The alkaloid or a derivative thereof may be in the form of a salt, particularly a pharmaceutically acceptable salt such as an acid addition salt. Examples of the pharmaceutically acceptable salt include addition salts of hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like inorganic acids and addition salts of acetic acid, succinic acid, malic acid, tartaric acid, citric acid, maleic acid, fumaric acid, methanesulfonic acid, p-toluenesulfonic acid and the like organic acids.

[0028] As a pharmaceutical preparation which contains alkaloids originated from a plant of the genus *Stephania*, Cepharanthin (registered trademark by Kaken Shoyaku Co., Ltd.) is already on the market as a preparation of alkaloids extracted from *Stephania cepharantha* Hayata.

[0029] Cepharanthin (registered trademark) contains cepharanthine, isotetrandrine, berbamine, cycleanine, homoaromoline, cepharanoline, aromoline, obamegine, norcycleanine, 2-norcepharanthine, 2-norcepharanoline, 2-norberbamine, secocepharanthine, obaberine, 2-norisotetrandrine, oxyacanthine and thalrugosine as alkaloids. Among these alkaloids, main alkaloid components of Cepharanthin (registered trademark) are cepharanthine, isotetrandrine, berbamine, cycleanine, homoaromoline and cepharanoline.

[0030] According to the present invention, the term "Cepharanthin (registered trademark)" means a pharmaceutical preparation of alkaloids extracted from *Stephania cepharantha* Hayata, which is an article on the market (available from Kaken Shoyaku Co., Ltd.), and the term "cepharanthine" means an alkaloid of the aforementioned structural formula (I) (in the formula, R_1 is CH_3, R_2 and R_3 form $-CH_2-$ and R_4 is CH_3).

[0031] The alkaloids originated from a plant belonging to the genus *Stephania* of the family *Menspermaceae*, derivatives thereof and salts thereof, as the active ingredient of the present invention, inhibit transcription of DNA having an NF-κB recognition sequence by inhibiting activity of the transcription factor NF-κB. Thus, said active ingredient can inhibit expression of corresponding protein of a gene effectively, if the gene has the NF-κB recognition sequence. In consequence, the NF-κB activity inhibitor, agent for the treatment and prevention of diseases upon which the NF-κB activity inhibiting action is effective and gene expression inhibitor of the present invention, which contain said active ingredient, can inhibit expression of genes of cytokines such as IL-1 and TNF, as well as interleukin-2 (to be referred to as IL-2 hereinafter in some cases), IL-6, IL-8, granulocyte colony stimulating factor (to be referred to as G-CSF hereinafter in some cases), interferon β (to be referred to as IFN-β hereinafter in some cases) and the like, genes of receptor antagonists of inflammatory cytokines such as interleukin-1 receptor antagonist (to be referred to as IL-IRA hereinafter in some cases) and the like, genes of major histocompatibility antigen (to be referred to as MHC hereinafter in some cases) class I, MHC class II, β2 microglobulin, immunoglobulin light chain, serum amyloid A, angiotensinogen, complement B, complement C4 and the like, C-myc gene which is one of oncogenes and genes of viruses such as human immunodeficiency virus (to be referred to as HIV hereinafter in some cases), simian virus 40 (to be referred to as SV40 hereinafter in some cases), cytomegalovirus (to be referred to as CMV hereinafter in some cases), adenovirus and the like, so that these activity inhibitor, therapeutic and preventive agent and expression inhibitor can prevent and treat diseases in which such genes are concerned and are particularly useful in preventing and treating inflammatory diseases, autoimmune diseases and viral diseases.

[0032] That is, the drug of the present invention is effective for the treatment and prevention of diseases such as rheumatoid arthritis, systemic lupus erythematosus, systemic scleroderma, Behcet disease, periarteritis, ulcerative colitis, Crohn disease, active chronic hepatitis, glomerular nephritis and the like various autoimmune diseases; and osteoarthritis, gout, atherosclerosis, psoriasis, atopic dermatitis, pulmonary diseases with granuloma, various intractable diseases in which inflammatory symptoms such as of various types of encephalitis are the basis of the morbid state, endotoxin shock, sepsis, inflammatory colitis, diabetes, acute myelocytic leukemia, pneumonia, heart transplantation,

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encephalomylitis, anorexia, acute hepatitis, chronic hepatitis, drug induced hepatic injury, alcoholic hepatitis, viral hepatitis, jaundice, hepatic cirrhosis, hepatic insufficiency, atrial myxoma, Castleman syndrome, multiple myeloma, Rennert T lymphomatosis, mesangial nephritis, renal cell carcinoma, cytomegaloviral hepatitis, cytomegaloviral retinopathy, adenoviral cold syndrome, adenoviral pharyngoconjunctival fever, adenoviral ophthalmia, AIDS and the like.

[0033] When the drug of the present invention is administered, said active ingredient may be used directly or by oral administration after making it into tablets, powders, granules, capsules, syrups and the like dosage forms, or by parenteral administration after making it into suppositories, injections, external preparations, drip infusions and the like dosage forms, but it is desirable to administer it as oral administration preparations.

[0034] Pharmaceutical preparations for use in the oral or parenteral administration are produced in the usual way using common pharmaceutically acceptable carriers. For example, when a solid preparation for oral administration use is prepared, the principal agent is mixed with a filler and, as occasion demands, a binder, a disintegrator, a lubricant, a coloring agent, a corrective and the like and then the mixture is made into tablets, coated tablets, granules, powders, capsules and the like forms. Lactose, corn starch, sucrose, glucose, sorbitol, crystalline cellulose, silicon dioxide or the like can be used as the filler, polyvinyl alcohol, polyvinyl ether, ethyl cellulose, methyl cellulose, acacia, tragacanth, gelatin, shellac, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, calcium citrate, dextrin, pectin or the like can be used as the binder, magnesium stearate, talc, polyethylene glycol, silica, hardened plant oil or the like can be used as the lubricant, pharmaceutically acceptable coloring agent can be used as the coloring agent, and cocoa powder, mentha water, aromatic acid, mentha oil, borneol, powdered cinnamon bark or the like can be used as the corrective. As a matter of course, these tablets and granules can be coated by sugar coating, gelatin coating and the like optional means as occasion demands. If necessary, an antiseptic agent, an antioxidant and the like may be added.

[0035] When injections, drip infusions and the like are prepared, a pH adjusting agent, a buffer, a stabilizer, a solubilizing agent and the like may be added to the principal agent and, after carrying out freeze drying or the like treatment as occasion demands, the mixture is made into injections or drip infusions for subcutaneous, intramuscular or intravenous administration use.

[0036] The subject of the present invention is vertebrates, preferably mammals, and more preferably human.

[0037] Dosage of the drug of the present invention varies depending on the kind of the disease, degree of the symptoms, age of the patient and the like conditions, but, when the drug is administered to human in the form of an oral preparation for example, it may be administered at a daily dose of generally from 0.02 to 20 mg/kg, preferably from 0.1 to 10 mg/kg, more preferably from 0.2 to 6 mg/kg, as said alkaloid, a derivative thereof or a salt thereof, by dividing the daily dose into 1 to several doses per day.

[0038] Since the active ingredient according to the present invention can inhibit NF-κB activity and also can inhibit expression of certain genes, for example, those having the NF-κB binding sequence (especially, the genes in which NF-κB is highly participated in their expression, such as HIV and TNF-α), it is apparent for one skilled in the art that the agents according to the present invention are useful in various *in vitro, in vivo, ex vivo* and other studies and experiments.

EXAMPLES

[0039] Examples of the present invention are given below by way of illustration and not by way of limitation.

Test Example 1

Inhibition action on NF-κB binding activation

[0040] In order to examine the action of a test compound cepharanthine to inhibit NF-κB binding activation, U1 [a cell strain derived from HIV-1 (HIV type 1) latent infection human monocyte] cells were stimulated or not stimulated with phorbol 12-myristate 13-acetate (PMA) in the presence or absence of the test compound, and the following assay was carried out using a nucleoprotein extract obtained from the resulting cells.

[Gel shift assay]

[0041] Gel shift assay was carried out using an NF-κB probe of HIV-1. A double-stranded DNA fragment of an NF-κB site-like sequence, 5'-AGT TGA GGG GAC TTT CCC AGG C-3' from the transcription initiation point, was used as the NF-κB probe of HIV-1. The 5'-terminus of the probe was radiation-labeled with ^{32}P in the usual way using [γ-^{32}P]ATP and polynucleotide kinase. In an ice bath, 10 μg of the nucleoprotein extract obtained in the above was mixed with a binding buffer [20 mM Hepes, pH 7.9/0.1 M KCl/0.5 mM dithiothreitol (DTT)/0.2 mM ethylenediaminetetraacetic acid (EDTA)/0.5 mM phenylmethylsulfonyl fluoride (PMSP)/20% glycerol] and, in order to detect only of the activity of the protein of interest in the nucleoprotein extract, further with DNA (carrier) poly(d1-dC) to which the protein of interest

does not bind, and the resulting mixture was allowed to undergo 5 minutes of the reaction at room temperature. At the same time, a sample in which a large quantity of non-labeled probe was added to the reaction solution was prepared and the same reaction was carried out as a competitive assay. Thereafter, the ^{32}P-labeled NF-κB probe was added thereto to carry out 20 minutes of binding reaction at room temperature. After the reaction, 4% unmodified polyacrylamide gel electrophoresis was carried out in order to separate the DNA-NF-κB complex from free oligonucleotide. The gel was subjected to autoradiography, and NF-κB in the nucleoprotein extract was determined by an image analyzer (BIO-RAD, Model GS-700 Imaging Densitometer).

[0042] The action of the test compound to inhibit activation of NF-κB binding by PMA stimulation of U1 cells (inhibition ratio, %) is shown in Table 1.

Table 1

	Average (OD)	Area (mm × mm)	Labeled amount (OD × mm × mm)	% Inhibition
V1	0.377	65.54	24.71	(-)
V2	0.473	74.85	35.40	-
V3	0.447	70.53	31.53	10.9*
V4	0.442	69.15	30.56	13.7*
V5	0.548	83.83	45.94	-
V6	0.491	70.45	34.59	24.7**
V7	0.490	50.63	24.81	46.0**
V8	0.377	54.39	20.51	55.4**
V9	0.447	80.61	36.03	21.6**

V1: Negative control (nucleoprotein extract not contained)
V2: Control (nucleoprotein extract of untreated U1 cells)
V3: Cepharanthine control; reaction (2.5 hours) of U1 cells in the presence of 0.1 μg/ml of cepharanthine
V4: Cepharanthine control; reaction (2.5 hours) of U1 cells in the presence of 1 μg/ml of cepharanthine
V5: Stimulation (0.5 hour) of U1 cells with 10 ng/ml of PMA
V6: Reaction (2 hours) of U1 cells in the presence of 0.1 μg/ml of cepharanthine + stimulation (0.5 hour) with 10 ng/ml of PMA
V7: Reaction (2 hours) of U1 cells in the presence of 1 μg/ml of cepharanthine + stimulation (0.5 hour) with 10 ng/ml of PMA
V8: (Competitive assay) V5 + competitive NF-κB cold probe
V9: (Competitive assay) V5 + noncompetitive SP1 cold probe
*: V3 and V4 vs. V2,
**: V6, V7, V8 and V9 vs. V5

[0043] The above results show that the drug of the present invention has significant effect to inhibit NF-κB binding activation.

Test Example 2

Inhibition action on HIV-1 LTR transcription activity

[0044] In order to examine effect of a test compound cepharanthine on the HIV-1 transcription activity, CAT assay was carried out by the following method.

[HIV-1 LTR CAT assay]

[0045] A plasmid in which chloramphenicol acetyltransferase (CAT) gene has been linked to the downstream of HIV-1 LTR gene was introduced by lipofection into HeLa cells adjusted to a density of 1×10^6 cells, and the resulting cells were cultured in the presence or absence of the test compound and phorbol 12-myristate 13-acetate (PMA). After 48

hours of the culturing, the cells were collected and washed to prepare a cell extract using a freeze-thawing method. The thus prepared cell extract was allowed to react with acetyl CoA and [^{14}C]-labeled chloramphenicol (CM), and the thus formed acetyl[^{14}C]CM was separated by a thin layer chromatography, subjected to autoradiography and then qualitatively measured using an image analyzer (BIO-RAD, Model GS-700 Imaging Densitometer). Inhibition ratio based on the labeled amount obtained in the absence of the test compound is shown in Table 2.

Table 2

	Average (OD)	Area (mm × mm)	Labeled amount (OD × mm × mm)	% Inhibition
V1	0.703	59.93	42.13	(-)
V2	0.905	72.02	65.18	(-)
V3	0.892	81.14	72.38	-
V4	0.892	54.31	48.44	33.1*
V5	0.867	41.00	35.55	50.9*

V1: Negative control
V2: Plasmid control
V3: HeLa cells were cultured in the presence of PMA (10 ng/ml)
V4: HeLa cells were cultured in the presence of cepharanthine (0.1 μg/ml) + PMA (10 ng/ml)
V5: HeLa cells were cultured in the presence of cepharanthine (1 μg/ml) + PMA (10 ng/ml)
*: V4 and V5 vs. V3

[0046] The above results show that the drug of the present invention has significant effect to inhibit HIV-1 LTR transcription activity.

Test Example 3

Acute toxicity test

[0047] The LD_{50} values (mg/kg) in an acute toxicity test carried out using male mice are shown in Table 3.

Table 3

Route of administration	Drugs tested	LD_{50} (mg/kg)
Oral	Cepharanthin (registered trademark)	1900
	cepharanthine	3410
Intravenous	Cepharanthin (registered trademark)	45
	cepharanthine	47.0
	berbamine	18.8
	isotetrandrine	32.5
	cycleanine	62.5
	homoaromoline	42.4
	cepharanoline	34.3

Formulation Example 1

[0048] A 500 mg portion of cepharanthine hydrochloride was thoroughly mixed with 3.0 g of lactose, 1.28 g of corn starch, 200 mg of hydroxypropyl cellulose and 20 mg of magnesium stearate, and the mixture was granulated and then made into tablets, thereby obtaining tablets of 100 mg per tablet.

Formulation Example 2

[0049] A 500 mg portion of an alkaloid fraction of *Stephania sasaki* Hayata was thoroughly mixed with 2.5 g of lactose, 1.75 g of potato starch, 240 mg of crystalline cellulose and 10 mg of calcium stearate, and the mixture was packed into capsules to prepare capsules each capsule containing 10 mg of alkaloid components.

Formulation Example 3

[0050] A 500 mg portion of an alkaloid fraction of *Stephania cepharantha* Hayata was dissolved in dilute hydrochloric acid, and the solution was mixed with distilled water for injection use, isotonized with sodium chloride and then filled up to a total volume of 100 ml. Thereafter, the resulting solution was filtered through a 0.2 μ membrane filter, dispensed and heat-sealed into 10 ml capacity ampoules and then heat-sterilized to obtain injections.

Production Example Production of Cepharanthin (registered trademark)

[0051] A methanol extract of tuberous roots of *Stephania cepharantha* Hayata belonging to the genus *Stephania* of the family *Menspermaceae* was dissolved in dilute hydrochloric acid, the resulting solution was alkalified with sodium hydroxide and then the thus formed precipitate was collected by filtration. The thus obtained precipitate was washed with dilute sodium hydroxide aqueous solution and extracted with ether, and then the resulting extract was concentrated under a reduced pressure to obtain the title product.

[0052] As has been described in the foregoing, the alkaloids originated from a plant belonging to the genus *Stephania* of the family *Menspermaceae*, derivatives thereof and salts thereof, as the active components of the present invention, exert an action to inhibit transcription of DNA having an NF-κB recognition sequence by inhibiting the activity of the transcription factor NF-κB. Because of this, the drug of the present invention which contains the just described active components can inhibit expression of genes of certain substances such as IL-1, TNF, IL-2, IL-6, IL-8, G-CSF, IFN-β and the like cytokines and IL-IRA and the like inflammatory cytokine receptor antagonists, as well as MHC class I, MHC class II, β2 microglobulin, immunoglobulin light chain, serum amyloid A, angiotensinogen, complement B, complement C4, C-myc gene, HIV, SV40, CMV, adenovirus and the like, so that the inventive drug is useful in treating and/or preventing various diseases, particularly inflammatory diseases, autoimmune diseases and viral diseases, in which these substances are taking roles.

[0053] While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof.

EDOCID: <EP_____0931544A2_I_>

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Kaken Shoyaku Co., Ltd.

(B) STREET: 3-37-10, Shimorenjaku, Mitaka-shi

(C) CITY: Tokyo

(E) COUNTRY: Japan

(F) POSTAL CODE (ZIP): 181-0013

(ii) TITLE OF INVENTION: NF-KB Activity Inhibitor

(iii) NUMBER OF SEQUENCES: 2

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:

APPLICATION NUMBER: EP 98 104 269.0

(vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: JP 9-353879

(B) FILING DATE: 22-DEC-1997

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GGGRNNYCCC 10

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double strand

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

AGTTGAGGGG ACTTTCCCAG GC 22

Claims

1. Use of at least one compound selected from the group consisting of alkaloids originated from a plant belonging to the genus *Stephania* of the family *Menspermaceae*, derivatives thereof and salts thereof for the preparation of a medicament for inhibiting NF-$_k$B activity.

2. Use according to claim 1, wherein the plant belonging to the genus *Stephania* is *Stephania cepharantha* Hayata.

3. Use of at least one compound selected from the group consisting of cepharanthine, isotetrandrine, berbamine, cycleanine, homoaromoline, cepharanoline, aromoline, obamegine, norcycleanine, 2-norcepharanthine, 2-norcepharanoline, 2-norberbamine, secocepharanthine, obaberine, 2-norisotetrandrine, oxyacanthine, stephibaberine, thalrugosine, coclaurine, reticuline, laudanidine, protosinomenine, N-methylcoclaurine, FK-3000, sinomenine, cephamonine, tannagine, cephamuline, lastourvilline, isocorydine, corydine, stepharine, cepharamine, aknadinine and aknadilactam, and derivatives thereof and salts thereof for the preparation of a medicament for inhibiting NF-

$_{k}$B activity.

4. Use according to claim 3, wherein the active ingredient is at least one compound selected from the group consisting of cepharanthine, isotetrandrine, berbamine, cycleanine, homoaromoline, and cepharanoline.

5. Use according to claim 4, wherein the active ingredient is cepharanthine.

6. Use of at least one compound selected from the group consisting of alkaloids originated from a plant belonging to the genus *Stephania* of the family *Menspermaceae*, derivatives thereof and salts thereof for the preparation of a medicament for the treatment and prevention of a disease upon which NF-$_{k}$B activity inhibiting action is effective.

7. Use according to claim 6, wherein the plant belonging to the genus *Stephania* is *Stephania cepharantha* Hayata.

8. Use of at least one compound selected from the group consisting of cepharanthine, isotetrandrine, berbamine, cycleanine, homoaromoline, cepharanoline, aromoline, obamegine, norcycleanine, 2-norcepharanthine, 2-norcepharanoline, 2-norberbamine, secocepharanthine, obaberine, 2-norisotetrandrine, oxyacanthine, stephibaberine, thalrugosine, coclaurine, reticuline, laudanidine, protosinomenine, N-methylcoclaurine, FK-3000, sinomenine, cephamonine, tannagine, cephamuline, lastourvilline, isocorydine, corydine, stepharine, cepharamine, aknadinine and aknadilactam, and derivatives thereof and salts thereof for the preparation of a medicament for treatment and prevention of a disease upon which NF-κB activity inhibiting action is effective.

9. Use according to claim 8, wherein the active ingredient is at least one compound selected from the group consisting of cepharanthine, isotetrandrine, berbamine, cycleanine, homoaromoline, and cepharanoline.

10. Use according to claim 9, wherein the active ingredient is cepharanthine.

11. Use according to any one of claims 6 or 8, wherein the disease upon which NF-$_{k}$B activity inhibiting action is effective is at least one disease selected from the group consisting of inflammatory diseases, autoimmune diseases and viral diseases.

12. Use according to any one of claims 1 to 3 wherein the inhibiting NF-$_{k}$B activity is manifested in the inhibition of expression for the gene of at least one member selected from the group consisting of interleukin-1 (IL-1), tumor necrosis factor (TNF), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-8 (IL-8), granulocyte colony stimulating factor (G-CSF), interferon β (IFN-β), interleukin-1 receptor antagonist (IL-IRA), major histocompatibility antigen (MHC) class I, major histocompatibility antigen (MHC) class II, β2 microglobulin, immunoglobulin light chain, serum amyloid A, angiotensinogen, complement B, complement C4, C-myc gene, human immunodeficiency virus (HIV), simian virus 40 (SV40), cytomegalovirus (CMV) and adenovirus.

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(54) NF-kB activity inhibitor

(57) This invention relates to an NF-κB activity inhibitor which contains alkaloids originated from a plant belonging to the genus *Stephania* of the family *Menspermaceae*, derivatives thereof and salts thereof, as the active components, to an agent for use in the treatment and prevention of diseases upon which the NF-κB activity inhibiting action is effective and to an inhibitor of the expression of related genes. Since said active components exert an action to inhibit transcription of DNA having an NF-κB recognition sequence by inhibiting the activity of NF-κB, the drug of the present invention can inhibit expression of genes of certain substances such as cytokines, inflammatory cytokine receptor antagonists, MHC class I, MHC class II, β2 microglobulin, immunoglobulin light chain, serum amyloid A, angiotensinogen, complement B, complement C4, C-myc gene, HIV, SV40, CMV, adenovirus and the like, so that the inventive drug is useful in treating and/or preventing various diseases in which these substances are taking roles.

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PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 98 10 4269

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	ONAI ET AL.: "Inhibitory Effects of Bisbenzylisochinoline Alkaloids on Induction of Proinflammatory Cytokines, Interleukin-1 and Tumor Necrosis Factor-.alpha." PLANTA MEDICA, vol. 61, 1995, pages 497-501, XP008007303 * abstract * * page 497, right-hand column, paragraph 4 * * page 498, left-hand column, paragraph 1 * * page 500, right-hand column, paragraph 2 *	1	A61K31/47 A61K31/485 A61K35/78
X	DATABASE MEDLINE [Online] August 1969 (1969-08), SHIMIZU M ET AL: "[Therapeutic effects of Cepharantine on herpes zoster]" XP002273710 Database accession no. NLM5393937 * abstract *	1	

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TECHNICAL FIELDS SEARCHED (Int.Cl6)

A61K

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
The Hague	17 March 2004	Langer, O

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C07)

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INCOMPLETE SEARCH
SHEET C

Application Number
EP 98 10 4269

Claim(s) searched completely:
-

Claim(s) searched incompletely:
1-12

Reason for the limitation of the search:

A) The expression "compound selected from the group consisting of alkaloids originated from a plant belonging to the genus Stephania of the family Menspermaceace, derivatives thereof and salts thereof" in claim 1 and dependent claims 2 and 12 lacks clarity under Article 84 EPC as it is unclear which chemical compounds are "originated" or obtainable from the above plant.
Furthermore, the expression "derivative" in claim 1 is unclear under Article 84 EPC, as it has not been specified how these derivatives have to be derived from the corresponding parent compounds, i.e. it is unclear which feature of these compounds is to be modified in order to obtain such derivatives.

B) Present claims 3, 6, 8 and dependent claims 4, 5, 7, 9 and 10 relate to a medicament for inhibiting NF-kappaB. Present claim 12 relates to the use of a medicament according to claims 1-3 of the present application for the inhibition of NF-kappaB that results in the inhibition of the expression for the gene of at least one member of the group consisting of interleukin-1 (IL-1), tumor necrosis factor (TNF), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-8 (IL-8), granulocyte colony stimulating factor (G-CSF), interferon beta (IFN-beta), interleukin-1 receptor antagonist (IL-IRA), major histocompatibility antigen (MHC) class I, major histocompatibility antigen (MHC) class II, beta2 microglobulin, immunoglobulin light chain, serum amyloid A, angiotensinogen, complement B, complement C4, C-myc gene, human immunodeficiency virus (HIV), simian virus 40 (SV40), cytomegalovirus (CMV) and adenovirus.
Such definitions of pathological conditions (diseases) by an underlying mechanism on a (bio)molecular basis, as in the present application, is considered to lead to a lack of clarity within the meaning of Article 84 EPC. It is impossible to compare the mechanistic characterising features (involvement of NF-kappaB and inhibition of an expression of the above genes) the applicant has chosen to employ with what is set out in the prior art (explicitly named pathological conditions, diseases) over the whole scope of subject-matter claimed. The lack of clarity is such as to render a meaningful complete search impossible.

C) Consequently, the search has been restricted to the (second) medical use of the alkaloids of the present application, as far as part of the first invention, for the treatment of those pathological conditions that are considered to be clear under Article 84 EPC and supported by the description, namely those explicitly mentioned in claim 11 and in the description on page 16, line 24 to page 17, line 18, as far as relating to the first invention, i.e. the use of at least one of the (7->8',11->12')-linked bisbenzylisoquinolines selected from the group consisting of 2-norcepharanoline, oxyacanthine, stephibaberine, 2-norcepharanthine, cepharanthine, cepharanoline, obaberine, homoaromoline, and aromoline in the manufacture of a medicament for

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INCOMPLETE SEARCH
SHEET C

Application Number
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inhibiting NF-kappa-B activity wherein the inhibition of NF-kappa-B activity serves to treat autoimmune diseases and inflammatory diseases, including rheumatoid arthritis, systemic lupus erythematosus, systemic scleroderma, Behcet disease, periarteritis, ulcerative colitis, Crohn disease, active chronic hepatitis, glomerular nephritis, osteoarthritis, gout, atherosclerosis, psoriasis, atopic dermatitis, pulmonary diseases with granuloma, encephalitis, endotoxin shock sepsis, inflammatory colitis, diabetes, acute myelocytic leukemia, pneumonia, heart transplantation, encephalomyelitis, anorexia, acute and chronic non-viral hepatitis, drug induced hepatic injury, alcoholic hepatitis, jaundice, hepatic cirrhosis, hepatic insufficiency, atrial myxoma, Castleman syndrome, multiple myeloma, Rennert T lymphomatosis, mesangial nephritis and renal cell carcinoma.

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PARTIAL EUROPEAN SEARCH REPORT

Application Number
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DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
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X	YASUKAWA K ET AL: "Bisbenzylisoquinoline alkaloids inhibit tumor promotion by 12-O-tetradecanoylphorbol-13-acetate in two-stage carcinogenesis in mouse skin." ONCOLOGY. SWITZERLAND 1993 MAR-APR, vol. 50, no. 2, March 1993 (1993-03), pages 137-140, XP008007629 ISSN: 0030-2414 * abstract * * page 137, left-hand column, line 1 - right-hand column, line 9 * * page 138, right-hand column, paragraph 1 * * page 139, left-hand column, paragraph 2 - page 140, right-hand column, paragraph 1 * * table 1 *	1-12	
X,D	PATENT ABSTRACTS OF JAPAN vol. 1997, no. 03, 31 March 1997 (1997-03-31) & JP 08 301761 A (KAKEN SHIYOUYAKU KK), 19 November 1996 (1996-11-19) * abstract *	1-12	
X	PATENT ABSTRACTS OF JAPAN vol. 018, no. 575 (C-1268), 4 November 1994 (1994-11-04) & JP 06 211661 A (KAKEN SHIYOUYAKU KK), 2 August 1994 (1994-08-02) * abstract *	1,3,4,6,8,9,11,12	

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TECHNICAL FIELDS SEARCHED (Int.Cl.6)

EPO FORM 1503 03.82 (P04C10)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 98 10 4269

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	PATENT ABSTRACTS OF JAPAN vol. 1997, no. 03, 31 March 1997 (1997-03-31) & JP 08 301778 A (KAKEN SHIYOUYAKU KK), 19 November 1996 (1996-11-19) * abstract *	1,3-6, 8-12	
X	US 4 235 890 A (DEBAT JACQUES ET AL) 25 November 1980 (1980-11-25) * claims 1,4,7 * * examples 2,3 *	1,3-6, 8-12	
X	WO 92/16226 A (SMITHKLINE BEECHAM CORP) 1 October 1992 (1992-10-01) * page 1, paragraph 1 - paragraph 2 * * page 4 * * page 5, line 10 * * claims 1,4,12 *	1,3-6, 8-12	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
X	US 5 627 195 A (HU SHIXING) 6 May 1997 (1997-05-06) * claim 1 * * column 1, paragraph 1 * * column 1, line 58 - column 2, line 5 * * column 2, line 19 - line 25 * * column 3, line 27 - line 51 *	1,3-6, 8-12	
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EPO FORM 1503 03.82 (P04C10)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 98 10 4269

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	THONG Y H ET AL: "Inhibitory effects of bisbenzylisoquinolines on synthesis of the inflammatory cytokines interleukin-1 and tumor necrosis factor-alpha" MEDIATORS OF INFLAMMATION, vol. 2, 1993, pages 199-203, XP008028607 ISSN: 0032-0943 * abstract * * page 199 - page 200, right-hand column, paragraph 1 * * page 201, right-hand column, last paragraph - page 202 * * figure 1 * * tables 1,2 *	1-12	
X	YOSHIOKA T ET AL: "EFFECT OF BILIRUBIN ON POTASSIUM RELEASE FROM CORD BLOOD ERYTHROCYTES" CELL STRUCTURE AND FUNCTION, vol. 5, no. 3, 1980, pages 209-214, XP008028668 ISSN: 0386-7196 * abstract *	1-12	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
X	SUZUKI M ET AL: "EFFECT OF CEPHARANTHIN ON PRODUCTION OF ACTIVE OXYGEN BY POLYMORPHONUCLEAR LEUKOCYTES" NISHINIHON JOURNAL OF DERMATOLOGY, vol. 48, no. 2, 1986, pages 278-283, XP008028662 ISSN: 0386-9784 * page 583 *	1-12	

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EPO FORM 1503 03.82 (P04C10)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 98 10 4269

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	SHICHIRI Y ET AL: "Intra-arterial infusion chemotherapy in combination with a biscoclaurine alkaloid, Cepharanthin, to treat bone metastasis arising from renal cell carcinoma." INTERNATIONAL JOURNAL OF UROLOGY: OFFICIAL JOURNAL OF THE JAPANESE UROLOGICAL ASSOCIATION. JAPAN DEC 1994, vol. 1, no. 4, December 1994 (1994-12), pages 349-351, XP008028664 ISSN: 0919-8172 * abstract *	1-12	
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EPO FORM 1503 03.82 (P04C10)

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Application Number
EP 98 10 4269

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	DATABASE WPI Section Ch, Week 199237 Derwent Publications Ltd., London, GB; Class B02, AN 1992-304657 XP002273712 & JP 04 210637 A (KAO CORP) 31 July 1992 (1992-07-31) * abstract * -----	1,3,4,6, 8,9,11, 12	
X	AKAMATSU H ET AL: "Effects of cepharanthin on neutrophil chemotaxis, phagocytosis, and reactive oxygen species generation." THE JOURNAL OF DERMATOLOGY. JAPAN NOV 1991, vol. 18, no. 11, November 1991 (1991-11), pages 643-648, XP008007628 ISSN: 0385-2407 * abstract * * page 643, left-hand column, line 1 - right-hand column, line 3 * * page 646, left-hand column, paragraph 5 - right-hand column, paragraph 3 * -----	1-12	TECHNICAL FIELDS SEARCHED (Int.Cl.6)

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EPO FORM 1503 03.82 (P04C10)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 98 10 4269

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	KONDO Y ET AL: "Inhibitory effect of bisbenzylisoquinoline alkaloids on nitric oxide production in activated macrophages." BIOCHEMICAL PHARMACOLOGY. ENGLAND 3 DEC 1993, vol. 46, no. 11, 3 December 1993 (1993-12-03), pages 1887-1892, XP008007637 ISSN: 0006-2952 * abstract * * page 1887, right-hand column, line 4 - line 5 * * page 1891, left-hand column, line 6 - line 13 *	1-12	
X	IVANOVSKA, NINA ET AL: "Study on the anti- inflammatory action of Berberis vulgaris root extract, alkaloid fractions and pure alkaloids" INTERNATIONAL JOURNAL OF IMMUNOPHARMACOLOGY (1997), VOLUME DATE 1996, 18(10), 553-561, XP002212064 * abstract * * page 553, left-hand column * * page 556 * * table 1 * * page 560, right-hand column, paragraph 2 - paragraph 3 *	1,3,6,8, 11,12	TECHNICAL FIELDS SEARCHED (Int.Cl.6)

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EPO FORM 1503 03.82 (P04C10)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 98 10 4269

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	MUELLER, K. ET AL: "The antipsoriatic Mahonia aquifolium and its active constituents;I. Pro- and antioxidant properties and inhibition of 5-lipoxygenase" PLANTA MED. (1994), 60(5), 421-4, XP008007581 * abstract * * page 421, right-hand column, paragraph 1 - paragraph 2 * * page 423, left-hand column, line 4 - page 424, left-hand column, paragraph 4 *	1,3,6,8, 11,12	
X	EP 0 806 204 A (SANKYO CO) 12 November 1997 (1997-11-12) * abstract * * examples 3,4 *	1,3-6, 8-12	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
X	BEZÁKOVÁ L ET AL: "Lipoxygenase inhibition and antioxidant properties of bisbenzylisoqunoline alkaloids isolated from Mahonia aquifolium." DIE PHARMAZIE. GERMANY OCT 1996, vol. 51, no. 10, October 1996 (1996-10), pages 758-761, XP001074319 ISSN: 0031-7144 * abstract * * page 758, right-hand column, line 1 - page 759, left-hand column, line 9 * * figures 1,2 * * page 760, left-hand column, paragraph 4 - right-hand column, paragraph 1 *	1,3,6,8, 11,12	

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EPO FORM 1503 03.82 (P04C10)

SDOCID: <EP_____0931544A3_I_>

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 98 10 4269

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
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A	FRETLAND D J ET AL: "RECENT ADVANCES IN LEUKOTRIENE B4 RECEPTOR ANTAGONIST RESEARCH" EXPERT OPINION ON THERAPEUTIC PATENTS, ASHLEY PUBLICATIONS, GB, vol. 6, no. 1, 1996, pages 21-28, XP000578976 ISSN: 1354-3776 * abstract * * page 21 * * page 22, left-hand column, paragraph 3 * * page 25, left-hand column, paragraph 2 *	1-12	TECHNICAL FIELDS SEARCHED (Int.Cl.6)

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EPO FORM 1503 03.82 (P04C10)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 98 10 4269

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
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X,P	LAMPERT M L ET AL: "Mahonia aquifolium" ZEITSCHRIFT FUR PHYTOTHERAPIE 1998 GERMANY, vol. 19, no. 2, 1998, pages 107-118, XP008028606 ISSN: 0722-348X * abstract * * page 107, column 3, last paragraph * * page 108, column 2, last paragraph - column 3, paragraph 5 * * page 109, column 1, paragraph 2 - column 2, paragraph 2 * * page 110, column 3, paragraph 2 - page 112, column 2, paragraph 1 * * page 113, column 1, paragraph 2 - column 2, paragraph 2 * * figures 3A,3B * * tables 2,4 * ---	1,3,6,8, 11,12	TECHNICAL FIELDS SEARCHED (Int.Cl.6)

EPO FORM 1503 03.82 (P04C10)

SDOCID: <EP_____0931544A3_I_>

European Patent Office

Application Number
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CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:

☒ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

1-12 (all partially)

European Patent Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 98 10 4269

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. claims: 1-12 (all partially)

Use of at least one of the (7->8',11->12')-linked bisbenzylisoquinolines selected from the group consisting of 2-norcepharanoline, oxyacanthine, stephibaberine, 2-norcepharanthine, cepharanthine, cepharanoline, obaberine, homoaromoline, and aromoline in the manufacture of a medicament for inhibiting NF-kappa-B activity wherein the inhibition of NF-kappa-B activity serves to treat autoimmune diseases and inflammatory diseases, including rheumatoid arthritis, systemic lupus erythematosus, systemic scleroderma, Behcet disease, periarteritis, ulcerative colitis, Crohn disease, active chronic hepatitis, glomerular nephritis, osteoarthritis, gout, atherosclerosis, psoriasis, atopic dermatitis, pulmonary diseases with granuloma, encephalitis, endotoxin shock sepsis, inflammatory colitis, diabetes, acute myelocytic leukemia, pneumonia, heart transplantation, encephalomyelitis, anorexia, acute and chronic non-viral hepatitis, drug induced hepatic injury, alcoholic hepatitis, jaundice, hepatic cirrhosis, hepatic insufficiency, atrial myxoma, Castleman syndrome, multiple myeloma, Rennert T lymphomatosis, mesangial nephritis and renal cell carcinoma.

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2. claims: 1-12 (all partially)

Use of at least one of the (7->8',11->12')-linked bisbenzylisoquinolines selected from the group consisting of 2-norcepharanoline, oxyacanthine, stephibaberine, 2-norcepharanthine, cepharanthine, cepharanoline, obaberine, homoaromoline, and aromoline in the manufacture of a medicament for inhibiting NF-kappa-B activity wherein the inhibition of NF-kappa-B activity serves to treat viral diseases, including viral hepatitis, cytomegaloviral hepatitis, cytomegaloviral retinopathy, adenoviral cold syndrome, adenoviral pharyngoconjunctival fever, adenoviral ophthalmia and AIDS, as far as not comprised within the first invention.

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3. claims: claims 1-4, 6-9, 11, 12 (all partially)

SDOCID: <EP_____0931544A3_I_>

European Patent Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 98 10 4269

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

Use of at least one of the (8->7',11->12')-linked bisbenzylisoquinolines selected from the group consisting of 2-norberbamine, 2-norisotetrandrine, thalrugosine, berbamine, isotetrandrine, and obamegine in the manufacture of a medicament for inhibiting NF-kappa-B activity wherein the inhibition of NF-kappa-B activity serves to treat autoimmune diseases and inflammatory diseases, including rheumatoid arthritis, systemic lupus erythematosus, systemic scleroderma, Behcet disease, periarteritis, ulcerative colitis, Crohn disease, active chronic hepatitis, glomerular nephritis, osteoarthritis, gout, atherosclerosis, psoriasis, atopic dermatitis, pulmonary diseases with granuloma, encephalitis, endotoxin shock sepsis, inflammatory colitis, diabetes, acute myelocytic leukemia, pneumonia, heart transplantation, encephalomyelitis, anorexia, acute and chronic non-viral hepatitis, drug induced hepatic injury, alcoholic hepatitis, jaundice, hepatic cirrhosis, hepatic insufficiency, atrial myxoma, Castleman syndrome, multiple myeloma, Rennert T lymphomatosis, mesangial nephritis and renal cell carcinoma, as far as not comprised within one of the preceding inventions.

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4. claims: claims 1-4, 6-9, 11, 12 (all partially)

Use of at least one of the (8->7',11->12')-linked bisbenzylisoquinolines selected from the group consisting of 2-norberbamine, 2-norisotetrandrine, thalrugosine, berbamine, isotetrandrine, and obamegine in the manufacture of a medicament for inhibiting NF-kappa-B activity wherein the inhibition of NF-kappa-B activity serves to treat viral diseases, including viral hepatitis, cytomegaloviral hepatitis, cytomegaloviral retinopathy, adenoviral cold syndrome, adenoviral pharyngoconjunctival fever, adenoviral ophthalmia and AIDS, as far as not comprised within one of the preceding inventions.

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5. claims: 1-4, 6-9, 11, 12 (all partially)

European Patent Office

LACK OF UNITY OF INVENTION SHEET B

Application Number
EP 98 10 4269

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

Use of at least one of the (8->12',12->8')-linked bisbenzylisoquinolines selected from the group consisting of norcycleanine, and cycleanine in the manufacture of a medicament for inhibiting NF-kappa-B activity wherein the inhibition of NF-kappa-B activity serves to treat autoimmune diseases and inflammatory diseases, including rheumatoid arthritis, systemic lupus erythematosus, systemic scleroderma, Behcet disease, periarteritis, ulcerative colitis, Crohn disease, active chronic hepatitis, glomerular nephritis, osteoarthritis, gout, atherosclerosis, psoriasis, atopic dermatitis, pulmonary diseases with granuloma, encephalitis, endotoxin shock sepsis, inflammatory colitis, diabetes, acute myelocytic leukemia, pneumonia, heart transplantation, encephalomyelitis, anorexia, acute and chronic non-viral hepatitis, drug induced hepatic injury, alcoholic hepatitis, jaundice, hepatic cirrhosis, hepatic insufficiency, atrial myxoma, Castleman syndrome, multiple myeloma, Rennert T lymphomatosis, mesangial nephritis and renal cell carcinoma, as far as not comprised within one of the preceding inventions.

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6. claims: 1-4, 6-9, 11, 12 (all partially)

Use of at least one of the (8->12',12->8')-linked bisbenzylisoquinolines selected from the group consisting of norcycleanine, and cycleanine in the manufacture of a medicament for inhibiting NF-kappa-B activity wherein the inhibition of NF-kappa-B activity serves to treat viral diseases, including viral hepatitis, cytomegaloviral hepatitis, cytomegaloviral retinopathy, adenoviral cold syndrome, adenoviral pharyngoconjunctival fever, adenoviral ophthalmia and AIDS, as far as not comprised within one of the preceding inventions.

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7. claims: 1-3, 6-8, 11, 12 (all partially)

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European Patent Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 98 10 4269

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

Use of the seco-bisbenzylisoquinoline secocepharantine in the manufacture of a medicament for inhibiting NF-kappa-B activity wherein the inhibition of NF-kappa-B activity serves to treat autoimmune diseases and inflammatory diseases, including rheumatoid arthritis, systemic lupus erythematosus, systemic scleroderma, Behcet disease, periarteritis, ulcerative colitis, Crohn disease, active chronic hepatitis, glomerular nephritis, osteoarthritis, gout, atherosclerosis, psoriasis, atopic dermatitis, pulmonary diseases with granuloma, encephalitis, endotoxin shock sepsis, inflammatory colitis, diabetes, acute myelocytic leukemia, pneumonia, heart transplantation, encephalomyelitis, anorexia, acute and chronic non-viral hepatitis, drug induced hepatic injury, alcoholic hepatitis, jaundice, hepatic cirrhosis, hepatic insufficiency, atrial myxoma, Castleman syndrome, multiple myeloma, Rennert T lymphomatosis, mesangial nephritis and renal cell carcinoma, as far as not comprised within one of the preceding inventions.

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8. claims: 1-3, 6-8, 11, 12 (all partially)

Use of the seco-bisbenzylisoquinoline secocepharantine in the manufacture of a medicament for inhibiting NF-kappa-B activity wherein the inhibition of NF-kappa-B activity serves to treat viral diseases, including viral hepatitis, cytomegaloviral hepatitis, cytomegaloviral retinopathy, adenoviral cold syndrome, adenoviral pharyngoconjunctival fever, adenoviral ophthalmia and AIDS, as far as not comprised within one of the preceding inventions.

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9. claims: claims 1-3, 6-8, 11, 12 (all partially)

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European Patent Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 98 10 4269

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

Use of at least one of the benzylisoquinolines selected from the group consisting of protosinomenine, N-methylcoclaurine, reticuline, coclaurine, and laudanidine in the manufacture of a medicament for inhibiting NF-kappa-B activity wherein the inhibition of NF-kappa-B activity serves to treat autoimmune diseases and inflammatory diseases, including rheumatoid arthritis, systemic lupus erythematosus, systemic scleroderma, Behcet disease, periarteritis, ulcerative colitis, Crohn disease, active chronic hepatitis, glomerular nephritis, osteoarthritis, gout, atherosclerosis, psoriasis, atopic dermatitis, pulmonary diseases with granuloma, encephalitis, endotoxin shock sepsis, inflammatory colitis, diabetes, acute myelocytic leukemia, pneumonia, heart transplantation, encephalomyelitis, anorexia, acute and chronic non-viral hepatitis, drug induced hepatic injury, alcoholic hepatitis, jaundice, hepatic cirrhosis, hepatic insufficiency, atrial myxoma, Castleman syndrome, multiple myeloma, Rennert T lymphomatosis, mesangial nephritis and renal cell carcinoma, as far as not comprised within one of the preceding inventions.

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10. claims: claims 1-3, 6-8, 11, 12 (all partially)

Use of at least one of the benzylisoquinolines selected from the group consisting of protosinomenine, N-methylcoclaurine, reticuline, coclaurine, and laudanidine in the manufacture of a medicament for inhibiting NF-kappa-B activity wherein the inhibition of NF-kappa-B activity serves to treat viral diseases, including viral hepatitis, cytomegaloviral hepatitis, cytomegaloviral retinopathy, adenoviral cold syndrome, adenoviral pharyngoconjunctival fever, adenoviral ophthalmia and AIDS, as far as not comprised within one of the preceding inventions.

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11. claims: claims 1-3, 6-8, 11, 12 (all partially)

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European Patent Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
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The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

Use of at least one of the morphinane derivatives selected from the group consisting of FK-3000, tannagine, cephamuline, and cephamonine in the manufacture of a medicament for inhibiting NF-kappa-B activity wherein the inhibition of NF-kappa-B activity serves to treat autoimmune diseases and inflammatory diseases, including rheumatoid arthritis, systemic lupus erythematosus, systemic scleroderma, Behcet disease, periarteritis, ulcerative colitis, Crohn disease, active chronic hepatitis, glomerular nephritis, osteoarthritis, gout, atherosclerosis, psoriasis, atopic dermatitis, pulmonary diseases with granuloma, encephalitis, endotoxin shock sepsis, inflammatory colitis, diabetes, acute myelocytic leukemia, pneumonia, heart transplantation, encephalomyelitis, anorexia, acute and chronic non-viral hepatitis, drug induced hepatic injury, alcoholic hepatitis, jaundice, hepatic cirrhosis, hepatic insufficiency, atrial myxoma, Castleman syndrome, multiple myeloma, Rennert T lymphomatosis, mesangial nephritis and renal cell carcinoma, as far as not comprised within one of the preceding inventions.

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12. claims: claims 1-3, 6-8, 11, 12 (all partially)

Use of at least one of the morphinane derivatives selected from the group consisting of FK-3000, tannagine, cephamuline, and cephamonine in the manufacture of a medicament for inhibiting NF-kappa-B activity wherein the inhibition of NF-kappa-B activity serves to treat viral diseases, including viral hepatitis, cytomegaloviral hepatitis, cytomegaloviral retinopathy, adenoviral cold syndrome, adenoviral pharyngoconjunctival fever, adenoviral ophthalmia and AIDS, as far as not comprised within one of the preceding inventions.

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13. claims: claims 1-3, 6-8, 11, 12 (all partially)

DOCID: <EP_____0931544A3_I_>

European Patent Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 98 10 4269

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

Use of at least one of the hasubanane derivatives selected from the group consisting of aknadilactam, aknadinine, cepharamine, and sinomenine in the manufacture of a medicament for inhibiting NF-kappa-B activity wherein the inhibition of NF-kappa-B activity serves to treat autoimmune diseases and inflammatory diseases, including rheumatoid arthritis, systemic lupus erythematosus, systemic scleroderma, Behcet disease, periarteritis, ulcerative colitis, Crohn disease, active chronic hepatitis, glomerular nephritis, osteoarthritis, gout, atherosclerosis, psoriasis, atopic dermatitis, pulmonary diseases with granuloma, encephalitis, endotoxin shock sepsis, inflammatory colitis, diabetes, acute myelocytic leukemia, pneumonia, heart transplantation, encephalomyelitis, anorexia, acute and chronic non-viral hepatitis, drug induced hepatic injury, alcoholic hepatitis, jaundice, hepatic cirrhosis, hepatic insufficiency, atrial myxoma, Castleman syndrome, multiple myeloma, Rennert T lymphomatosis, mesangial nephritis and renal cell carcinoma, as far as not comprised within one of the preceding inventions.

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14. claims: claims 1-3, 6-8, 11, 12 (all partially)

Use of at least one of the hasubanane derivatives selected from the group consisting of aknadilactam, aknadinine, cepharamine, and sinomenine in the manufacture of a medicament for inhibiting NF-kappa-B activity wherein the inhibition of NF-kappa-B activity serves to treat viral diseases, including viral hepatitis, cytomegaloviral hepatitis, cytomegaloviral retinopathy, adenoviral cold syndrome, adenoviral pharyngoconjunctival fever, adenoviral ophthalmia and AIDS, as far as not comprised within one of the preceding inventions.

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15. claims: claims 1-3, 6-8, 11, 12 (all partially)

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European Patent Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 98 10 4269

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

Use of at least one of the dibenzo[de,g]-quinoline derivatives selected from the group consisting of lastourvilline, corydine, and isocorydine in the manufacture of a medicament for inhibiting NF-kappa-B activity wherein the inhibition of NF-kappa-B activity serves to treat autoimmune diseases and inflammatory diseases, including rheumatoid arthritis, systemic lupus erythematosus, systemic scleroderma, Behcet disease, periarteritis, ulcerative colitis, Crohn disease, active chronic hepatitis, glomerular nephritis, osteoarthritis, gout, atherosclerosis, psoriasis, atopic dermatitis, pulmonary diseases with granuloma, encephalitis, endotoxin shock sepsis, inflammatory colitis, diabetes, acute myelocytic leukemia, pneumonia, heart transplantation, encephalomyelitis, anorexia, acute and chronic non-viral hepatitis, drug induced hepatic injury, alcoholic hepatitis, jaundice, hepatic cirrhosis, hepatic insufficiency, atrial myxoma, Castleman syndrome, multiple myeloma, Rennert T lymphomatosis, mesangial nephritis and renal cell carcinoma, as far as not comprised within one of the preceding inventions.

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16. claims: claims 1-3, 6-8, 11, 12 (all partially)

Use of at least one of the dibenzo[de,g]-quinoline derivatives selected from the group consisting of lastourvilline, corydine, and isocorydine in the manufacture of a medicament for inhibiting NF-kappa-B activity wherein the inhibition of NF-kappa-B activity serves to treat viral diseases, including viral hepatitis, cytomegaloviral hepatitis, cytomegaloviral retinopathy, adenoviral cold syndrome, adenoviral pharyngoconjunctival fever, adenoviral ophthalmia and AIDS, as far as not comprised within one of the preceding inventions.

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17. claims: claims 1-3, 6-8, 11, 12 (all partially)

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European Patent Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 98 10 4269

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, *namely*:

Use of stepharine in the manufacture of a medicament for inhibiting NF-kappa-B activity wherein the inhibition of NF-kappa-B activity serves to treat autoimmune diseases and inflammatory diseases, including rheumatoid arthritis, systemic lupus erythematosus, systemic scleroderma, Behcet disease, periarteritis, ulcerative colitis, Crohn disease, active chronic hepatitis, glomerular nephritis, osteoarthritis, gout, atherosclerosis, psoriasis, atopic dermatitis, pulmonary diseases with granuloma, encephalitis, endotoxin shock sepsis, inflammatory colitis, diabetes, acute myelocytic leukemia, pneumonia, heart transplantation, encephalomyelitis, anorexia, acute and chronic non-viral hepatitis, drug induced hepatic injury, alcoholic hepatitis, jaundice, hepatic cirrhosis, hepatic insufficiency, atrial myxoma, Castleman syndrome, multiple myeloma, Rennert T lymphomatosis, mesangial nephritis and renal cell carcinoma, as far as not comprised within one of the preceding inventions.

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18. claims: claims 1-3, 6-8, 11, 12 (all partially)

Use of stepharine in the manufacture of a medicament for inhibiting NF-kappa-B activity wherein the inhibition of NF-kappa-B activity serves to treat viral diseases, including viral hepatitis, cytomegaloviral hepatitis, cytomegaloviral retinopathy, adenoviral cold syndrome, adenoviral pharyngoconjunctival fever, adenoviral ophthalmia and AIDS, as far as not comprised within one of the preceding inventions.

SDOCID: <EP_____0931544A3_I_>

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 98 10 4269

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

17-03-2004

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